Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) constitute the BCR-ABL1–negative myeloproliferative neoplasms and are characterized by mutually exclusive Janus kinase 2 (JAK2), calreticulin (CALR), and myeloproliferative leukemia virus oncogene (MPL) mutations; respective frequencies of these mutations are approximately 95%, 0%, and 0% in PV, 60%, 20%, and 3% in ET, and 60%, 25%, and 7% in PMF. These mutations might be accompanied by other mutations that are less specific to myeloproliferative neoplasms but are prognostically relevant, such as additional sex combs–like 1 (ASXL1). Characteristic bone marrow morphology is required for World Health Organization–compliant diagnosis, especially in distinguishing ET from prefibrotic PMF and masked PV. Survival is the longest in ET, although still inferior to that of the age- and sex-matched control population; median survivals for patients younger than 60 years are approximately 33 years for ET, 24 for PV, and 15 for PMF. Major disease complications include thrombosis and leukemic or fibrotic transformation. In PV and ET, risk factors for survival include older age, leukocytosis, and thrombosis, whereas JAK2 mutation in ET is associated with increased risk of thrombosis. In PMF, type 1 or type 1–like CALR mutations are associated with superior and ASXL1 with inferior survival. Prevention of thrombosis in PV is secured by phlebotomy (hematocrit target <45%) and in both PV and ET by low-dose aspirin therapy; high-risk patients derive additional antithrombotic benefit from cytoreductive therapy with hydroxyurea as first-line and interferon–alfa and busulfan as second-line drugs of choice. Although the JAK inhibitor ruxolitinib was recently approved for use in hydroxyurea-resistant PV, its role in routine clinical practice remains debatable. In myelofibrosis, stem cell transplant is the current treatment of choice for genetically or clinically high-risk disease; for all other patients requiring treatment, participation in clinical trials may be preferred because currently available drugs, including JAK inhibitors, are palliative and not shown to be disease modifying.

In early 2014, the World Health Organization (WHO) committee for the classification of hematopoietic tumors was summoned in Chicago, Illinois, to revise its 2008 document, including the classification of chronic myeloid neoplasms (Figure 1). In regards to the diagnostic criteria for the BCR-ABL1–negative myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), a premeeting proposal from key committee members underscored the importance of bone marrow morphology in the diagnosis of ET, especially in distinguishing it from “prefibrotic PMF” and “masked PV.” The latter are respectively characterized by a bone marrow morphology that is consistent with PMF and PV but without overt fibrosis (prefibrotic PMF) and hemoglobin concentrations less than the WHO threshold levels of 18.5 g/dL in men and 16.5 g/dL in women (masked PV) (to convert to grams per liter, multiply by 10.0). The particular proposal also highlighted the increasing role of novel mutations, including the most recently discovered calreticulin (CALR) mutation, in both diagnosis and disease prognostication. The discovery of new mutations in MPN has also facilitated the development of molecularly targeted therapy, including (Janus kinase) JAK inhibitors, which have shown promising activity in controlling constitutional symptoms and splenomegaly in MF and PV. The present review focuses on (1) the diagnostic interface between bone marrow morphology and mutations in MPN, (2) clinical and genetic models of disease prognostication, and (3) risk-based treatment approaches in ET, PV, and MF.

Mutations

In the past 10 years, several somatic mutations have been described in MPN and can be operationally classified into MPN “specific” and “non-specific” (Table). The former are often mutually exclusive and include JAK2 (located on chromosome 9p24), CALR (calreticulin; located on chromosome 1p34-35), and MPL (myeloproliferative leukemia virus oncogene; located on chromosome 1p34). JAK2 is the most frequent, with frequencies of approximately 98% in PV, 50% to 60% in ET, and 55% to 65% in PMF. With the exception of rare reports, CALR and MPL mutations are absent in PV and their frequencies are approximately 20% to 25% and 3% to 4%, respectively, in ET and 20% to 25% and
6% to 7% in PMF. Approximately 10% to 15% of patients with PMF or ET do not express any of the 3 mutations and are referred to as being “triple negative.” Of note, a rare mutation involving LNK (a membrane-bound adaptor protein that is a negative regulator of JAK2 signaling) has been described in some JAK2 mutation–negative cases of PV and is believed to result in similar functional consequences. JAK2 mutations have been reported in other myeloid neoplasms, including refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T; approximately 50% frequency). There are significant differences in phenotype among the JAK2, CALR, and MPL mutational categories; JAK2 mutations are generally associated with older age, higher hemoglobin level, leukocytosis, lower platelet count, and increased risk of thrombosis; a higher JAK2 mutant allele burden with pruritus and fibrotic transformation in PV; mutant CALR in ET with younger age, male sex, higher platelet count, lower hemoglobin level, lower leukocyte count, and lower incidence of thrombotic events; and mutant CALR in PMF with younger age, higher platelet count, and lower frequencies of anemia, leukocytosis, and spicule some mutations. Furthermore, more than 80% of patients with mutant CALR harbor 1 of 2 mutation variants: type 1, a 52-bp deletion (p.L367fs*46), or type 2, a 5-bp TTGTC insertion (p.K385fs*47). In ET, type 2 CALR mutation was associated with significantly higher platelet count and, in PMF, with higher Dynamic International Prognostic Scoring System (DIPSS)–plus score, circulating blast percentage, and leukocyte count and inferior survival. Non-type 1 or type 2 CALR mutations are operationally classified into “type 1–like” and “type 2–like” variants on the basis of their structural similarities to type 1 and type 2 CALR variants, respectively, which is in turn based on α-helix content of the mutant C-terminus.

The Table lists additional MPN-nonspecific mutations, whose frequency is often greater in PMF, compared with PV or ET, among these, and in PMF, with higher Dynamic International Prognostic Scoring System (DIPSS)–plus score, circulating blast percentage, and leukocyte count and inferior survival. Non-type 1 or type 2 CALR mutations are operationally classified into “type 1–like” and “type 2–like” variants on the basis of their structural similarities to type 1 and type 2 CALR variants, respectively, which is in turn based on α-helix content of the mutant C-terminus.
those with mutational frequencies of 10% or more in PMF include ASXL1 (additional sex combs-like 1), TET2 (TET oncogene family member 2), SRSF2 (serine/arginine-rich splicing factor 2), and U2AF1 (U2 small nuclear RNA auxiliary factor 1). Other mutations that are less frequent in chronic-phase disease but with significantly higher frequency in blast-phase MPN include IDH1 and IDH2 (isocitrate dehydrogenase 1 and 2), TP53 (tumor protein p53), DNMT3A (DNA cytosine methyltransferase 3a), IKZF1 (IKAROS family zinc finger 1), JAK2, Janus kinase 2; MPL, myeloproliferative leukemia virus oncogene; SETBP1, SET binding protein 1; SF3B1, splicing factor 3B subunit 1; SRSF2, serine/arginine-rich splicing factor; TET2, TET oncogene family member; TP53, tumor protein p53.

Pathogenesis

Given the fact that MPN constitutes a stem cell–derived clonal myeloproliferation that has the potential to degenerate into acute myeloid leukemia (AML) or MF, it is reasonable to assume the contribution of somatic mutations in both clonal origination and evolution. It is reasonable to assume that some of the aforementioned MPN-specific mutations might be sufficient but not necessarily essential in initiating the disease process. The particular concept is in part supported by a plethora of “MPN” mouse models attached to a spectrum of directly MPN-relevant (eg, JAK2, MPL, and CALR) and not so relevant (eg, TP53, NRAS, FLT3, SHP-2, NF-E2) mutations. One also needs to explain how single mutations result in different WHO-defined clinicopathologic entities, although differences in mutant allele burden (mutation homozygosity), STAT1 (signal transducers and activators of transcription 1) signaling, order of mutation acquisition, and clonal heterogeneity have been considered as possible explanations in this regard.

JAK2 and MPL mutations are believed to directly activate JAK-STAT and make myeloproliferation cytokine independent or hyper-sensitive. The same scenario is assumed with LNK and CBL loss-of-function mutations that are believed to abrogate negative regulation of JAK-STAT. The precise mechanism of mutant CALR-induced myeloproliferation is less clear, but mouse models have supported a primary effect on platelet production. Recent communications suggest the central role of JAK-STAT activation in MPN, but the particular concept is confounded by the coexistence of an inflammatory state in MPN with aberrant cytokine expression and the fact that activated JAK-STAT is a nonspecific common phenomenon in cancer. Furthermore, targeted therapy with JAK inhibitors has so far failed to induce selective suppression of the disease clone in MPN.

New data are now emerging regarding the concomitant presence of other mutations in JAK2-, MPL-, or CALR-mutated MPN. The pathogenetic role of these other mutations is much less understood but believed to involve cooperation with the aforementioned driver mutations (eg, JAK2, MPL, CALR), which primarily affect cytokine signaling, in order to further disrupt epigenetic (eg, ASXL1, TET2, EZH2, IDH1, IDH2, DNMT3A), RNA splicing (eg, SRSF2, U2AF1, SF3B1), or transcriptional (TP53, IKZF1, NF-E2, CUX1) regulation. The higher prevalence of some of these mutations in blast-phase MPN suggests a possible role in disease progression or transformation into AML; such a possibility was recently demonstrated in compound mutant mouse models in which expression of JAK2V617F with TET2 loss induced disease progression and, with TP53 mutations, overt

Table. Mutations in Myeloproliferative Neoplasms (MPNs)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Chromosome Location</th>
<th>Approximate Mutational Frequency, %</th>
<th>Primary Myelofibrosis</th>
<th>Blast-Phase MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific to MPN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td>9p24</td>
<td>95</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Exon 14 mutation; ie, JAK2V617F</td>
<td>9p24</td>
<td>95</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Exon 12 mutations</td>
<td>9p24</td>
<td>95</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>CALR Exon 9 deletions and insertions</td>
<td>19p13.2</td>
<td>3</td>
<td>Infrequent</td>
<td>Infrequent</td>
</tr>
<tr>
<td>MPL Exon 10 mutations</td>
<td>1p34</td>
<td>Infrequent</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>LNK (as in Links; aka SHZB3) Exon 2 mutations</td>
<td>12q24.12</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>Infrequent</td>
</tr>
<tr>
<td>Non-specific to MPN</td>
<td>4q24</td>
<td>16</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>ASXL1 Exon 12 mutations</td>
<td>20q11.1</td>
<td>7</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>IDH1/IDH2 Exon 4 mutations</td>
<td>1p32.31</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>EZH2 Mutations involve several exons</td>
<td>7q36.1</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>5</td>
</tr>
<tr>
<td>DNMT3A Most frequent mutations affect amino acid R882</td>
<td>2p23</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>CBL Exon 8/9 mutations</td>
<td>11q23.3</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>4</td>
</tr>
<tr>
<td>IKZF1 Mostly deletions including intragenic</td>
<td>7p12</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>Infrequent</td>
</tr>
<tr>
<td>TP53 Exons 4 through 9</td>
<td>17p13.1</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>4</td>
</tr>
<tr>
<td>SF3B1 Exons 14 and 15, mostly</td>
<td>2q33.1</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>7</td>
</tr>
<tr>
<td>SRSF2 Exon 2</td>
<td>17q25.1</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>17</td>
</tr>
<tr>
<td>U2AF1</td>
<td>21q22.3</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>16</td>
</tr>
<tr>
<td>SETBP1 Exon 4</td>
<td>18q21.1</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Abbreviations: ASXL1, additional sex combs-like 1; CALR, calreticulin; CBL, casitas B-lineage lymphoma proto-oncogene; DNMT3A, DNA cytosine methyltransferase; EZH2, enhancer of zeste homologue 2; IDH1/IDH2, isocitrate dehydrogenase; IKZF1, IKAROS family zinc finger 1; JAK2, Janus kinase 2; MPL, myeloproliferative leukemia virus oncogene; SETBP1, SET binding protein 1; SF3B1, splicing factor 3B subunit 1; SRSF2, serine/arginine-rich splicing factor; TET2, TET oncogene family member; TP53, tumor protein p53.
regarding their precise pathogenetic contribution.24,25 Even more citations in “normal” elderly individuals has added to the complexity cell clonal distribution, and sensitivity to specific therapy.26 
multiplemutations was shown to influence disease phenotype, stem recently, the order of mutation acquisition in patients with MPN with enough to warrant genetical screening of unaffected family members.27 
sidering a myeloid neoplasm. Furthermore, a sound clinical assess-
ment is advised before embarking on mutation screening, especially in cases of thrombocytosis, which might accompany iron deficiency, the post-splenectomy or postsurgery state, infections, inflammatory disorders, hemolysis, trauma, and other conditions.

The differential diagnosis of erythrocytosis includes PV but also congenital or secondary erythrocytosis.28 Polycythemia vera is almost always associated with a JAK2 mutation, and its absence makes its diagnosis unlikely but not impossible; rare cases of JAK2 mutation-negative PV associated with LNK12 or CALR10 mutations have been reported. However, such cases are often associated with subnormal serum erythropoietin level, and therefore, the combination of JAK2 mutation screening and serum erythropoietin measurement should enable capturing almost all cases of PV (Figure 2).29

Clonal markers for the evaluation of thrombocytosis include JAK2V617F (present in approximately 50% of patients with ET or PMF), CALR mutations (present in approximately 20% to 25% of patients with ET or PMF), and MPL mutations (present in 3%-7% of patients with ET or PMF, respectively); in addition, thrombocytosis might accompany chronic myeloid leukemia, which should be addressed by BCR-ABL1 mutation screening, and RARS-T, which should be addressed by SF3B1 mutation screening.30 The hierarchy of peripheral blood mutation screening should be JAK2V617F first, and if results are negative, CALR, followed if negative by MPL (Figure 2). A bone marrow examination might be needed if blood samples are negative for all of these mutations, in order to morphologically distinguish clonal from reactive thrombocytosis and, if results are positive, to make accurate diagnosis of the underlying myeloid neoplasm (Figure 2).

<table>
<thead>
<tr>
<th>PV diagnosis unlikely if no JAK2 mutation and serum erythropoietin level normal or high</th>
<th>Bone marrow examination advised to confirm diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or high</td>
<td>Subnormal</td>
</tr>
<tr>
<td>Diagnosis possible</td>
<td>Diagnosis likely</td>
</tr>
<tr>
<td>JAK2V617F mutation screen</td>
<td>V617F mutation screen</td>
</tr>
<tr>
<td>Exon 12 mutation screen</td>
<td></td>
</tr>
<tr>
<td>Obtain serum erythropoietin level</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of PMF considered if bone marrow morphology is consistent with PMF and</td>
<td></td>
</tr>
<tr>
<td>1. mutation of JAK2, CALR, or MPL is present; or</td>
<td></td>
</tr>
<tr>
<td>2. trisomy 9 or del(13q) is present; or</td>
<td></td>
</tr>
<tr>
<td>3. other malignant neoplasms are excluded</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of PV considered if no JAK2 mutation and serum erythropoietin level normal or high</td>
<td>Bone marrow examination (required) to confirm diagnosis and distinguish ET from prefibrotic PMF</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Perform peripheral blood examination</td>
<td>Perform peripheral blood examination</td>
</tr>
<tr>
<td>JAK2V617F mutation screen</td>
<td>CALR mutation screen</td>
</tr>
<tr>
<td>MPL mutation screen</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Diagnosis of ET considered if bone marrow morphology is consistent with ET and</td>
<td></td>
</tr>
<tr>
<td>1. mutation of JAK2, CALR, or MPL is present; or</td>
<td></td>
</tr>
<tr>
<td>2. trisomy 9 or del(13q) is present; or</td>
<td></td>
</tr>
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<td>3. other malignant neoplasms are excluded</td>
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<td></td>
</tr>
<tr>
<td>3. other malignant neoplasms are excluded</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2. Practical Algorithm for Diagnosis of Polycythemia Vera (PV), Essential Thrombocytemia (ET), and Primary Myelofibrosis (PMF)**

AML.17 The recent demonstration of TET2, ASXL1, and DNMT3A mutations in “normal” elderly individuals has added to the complexity regarding their precise pathogenetic contribution.24,25 Even more recently, the order of mutation acquisition in patients with MPN with multiple mutations was shown to influence disease phenotype, stem cell clonal distribution, and sensitivity to specific therapy.26

**Diagnosis**

**When Should One Suspect MPN?** It is reasonable to consider the possibility of MPN in the presence of a complete blood count abnormality that exceeds the upper limit of the reference range or is associated with leukoerythroblastic smear; in this regard, erythrocytosis and thrombocytosis, respectively, suggest PV and ET whereas leukoerythroblastic smear is one of the cardinal features of MF. Myeloproliferative neoplasm should also be suspected in the presence of MPN-characteristic features such as palpable splenomegaly, anemia, aquagenic pruritus, unusual thrombosis such as portal or hepatic vein thrombosis, bone marrow fibrosis, and extramedullary hematopoiesis. Conversely, although MPN risk is higher in first-degree relatives of affected patients,27 the effect is not large enough to warrant genetic screening of unaffected family members.

**Distinguishing Clonal From Reactive or Secondary Erythrocytosis or Thrombocytosis**

In routine clinical practice, one has to first exclude the possibility of reactive or secondary erythrocytosis or thrombocytosis before considering a myeloid neoplasm. Furthermore, a sound clinical assessment is advised before embarking on mutation screening, especially in cases of thrombocytosis, which might accompany iron deficiency, the post-splenectomy or postsurgery state, infections, inflammatory disorders, hemolysis, trauma, and other conditions.

The differential diagnosis of erythrocytosis includes PV but also congenital or secondary erythrocytosis.28 Polycythemia vera is almost always associated with a JAK2 mutation, and its absence makes its diagnosis unlikely but not impossible; rare cases of JAK2 mutation-negative PV associated with LNK12 or CALR10 mutations have been reported. However, such cases are often associated with subnormal serum erythropoietin level, and therefore, the combination of JAK2 mutation screening and serum erythropoietin measurement should enable capturing almost all cases of PV (Figure 2).29

Clonal markers for the evaluation of thrombocytosis include JAK2V617F (present in approximately 50% of patients with ET or PMF), CALR mutations (present in approximately 20% to 25% of patients with ET or PMF), and MPL mutations (present in 3%-7% of patients with ET or PMF, respectively); in addition, thrombocytosis might accompany chronic myeloid leukemia, which should be addressed by BCR-ABL1 mutation screening, and RARS-T, which should be addressed by SF3B1 mutation screening.30 The hierarchy of peripheral blood mutation screening should be JAK2V617F first, and if results are negative, CALR, followed if negative by MPL (Figure 2). A bone marrow examination might be needed if blood samples are negative for all of these mutations, in order to morphologically distinguish clonal from reactive thrombocytosis and, if results are positive, to make accurate diagnosis of the underlying myeloid neoplasm (Figure 2).
Differential Diagnosis of Clonal Myeloproliferation

Almost all patients with PV harbor a JAK2 mutation (96% display the exon 14 JAK2V617F and 3% the exon 12 of JAK2 mutation). However, JAK2V617F is also present in approximately half of patients with ET or PMF, including those with "prefibrotic PMF." Similarly, CALR mutations occur in both ET (15%-24% incidence) and PMF (25%-35% incidence). MPL mutations occur in approximately 4% of patients with ET and 8% of patients with PMF. Therefore, mutation screening alone is not adequate to make WHO-compliant diagnosis of ET, PV, PMF, or prefibrotic PMF and a bone marrow morphological review is accordingly required (Figure 2).

In PV, the bone marrow is characterized by trilineage myeloproliferation with pleomorphic megakaryocytes, in ET by megakaryocyte proliferation with large and mature morphology, and in PMF by megakaryocyte proliferation and atypia (ie, small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering) accompanied by either reticulin and/or collagen fibrosis; in prefibrotic PMF, the megakaryocyte changes typically seen in PMF are accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis. 31

The 2008 WHO diagnostic criteria for PV, ET, and PMF are currently undergoing revision. 2 Expected changes in PV include lowering of the diagnostic hemoglobin/hematocrit level to 16.5 g/dL/49% in men and 16 g/dL/48% in women, in the presence of consistent bone marrow morphology, and the inclusion of bone marrow morphology as a major criterion, along with JAK2 mutation screening. 2

In ET and PMF, the proposed changes included the inclusion of CALR mutations as a clonal marker. Figure 2 represents a genetic-morphology combined diagnostic algorithm for PV, ET, and PMF.

Prognosis

Polycythemia Vera and Essential Thrombocythemia

In general, life expectancy in MPNs is inferior to that of the age- and sex-matched control population and median survival is approximately 20 years for ET, 14 years for PV, and 6 years for PMF. 3 In patients younger than 60 years, the corresponding median survival rates are approximately 33, 24, and 15 years. 8 Risk factors for survival in ET and PV include advanced age, leukocytosis and thrombosis, 32-34 and abnormal karyotype in PV 23; cytogenetic studies are recommended in PV at time of diagnosis and are expected to have abnormal results in approximately 12% of patients. Accordingly, in PV, the absence of age older than 70 years, leucocyte count greater than 13 000/µL (to convert to ×10⁹ per liter, multiply by 0.001), and thrombosis was associated with a 10-year relative survival of 84% vs 59% in the presence of and 26% with or more of these risk factors. 34 In ET, the absence of age at least 60 years, hemoglobin concentration below normal value, and leucocyte count greater than 15 000/µL was associated with a median survival of more than 20 years whereas the presence of 2 or more of these risk factors was associated with a median survival of only 9 years. 34 JAK2/CALR mutational status or JAK2V617F allele burden have not been shown to affect survival in either ET or PV. 5

Leukemic transformation rates at 20 years are estimated at less than 10% for PV and 5% for ET; fibrotic transformation rates are slightly higher. Risk factors for leukemic transformation in PV include advanced age, leukocytosis, and abnormal karyotype, 32 and for fibrotic transformation JAK2V617F allele burden of more than 50%. 36 In routinely diagnosed ET that includes cases of prefibrotic PMF, overall survival was adversely affected by prefibrotic PMF morphology, advanced age, thrombosis history, leukocytosis, and anemia; leukemia-free survival by prefibrotic PMF morphology, thrombosis, and extreme thrombocytosis (platelet count >1000 × 10³/µL [to convert to ×10⁹ per liter, multiply by 1.0]); and fibrosis-free survival by prefibrotic PMF morphology, advanced age, anemia, and absence of JAK2V617F. 37

Risk factors for arterial thrombosis in PV include previous arterial events and hypertension and, for venous thrombosis, previous venous events and older age. 38 Risk factors for arterial thrombosis in WHO-defined ET include age older than 60 years, thrombosis history, cardiovascular risk factors, leukocytosis, and presence of JAK2V617F, and for venous thrombosis male sex. 39 Risk factors for thrombosis in ET were not further modified by CALR mutations. 40 Regardless, cytoreductive treatment-relevant risk stratification in both ET and PV is currently based on only two factors (age ≥60 years and history of thrombosis): low risk (0 risk factors) and high risk (1 or 2 risk factors) (Figure 3). However, individualized therapy considers other risk factors and the risk of bleeding associated with extreme thrombocytosis (see Treatment subsection and Figure 3). 41,42

Primary Myelofibrosis

The most comprehensive prognostic model in PMF is the DIPSS-plus, which uses 8 adverse features: age older than 65 years, hemoglobin concentration less than 10 g/dL, leukocyte count greater than 25 000/µL, circulating blasts at least 1%, presence of constitutional symptoms, unfavorable karyotype (ie, complex karyotype or sole or 2 abnormalities that include +8, -7/-7q, +17q, inv(3), -5/5q, -12p, or +11q23 rearrangement), red cell transfusion need, and platelet count less than 100 × 10³/µL; 42 the 4 DIPSS-plus risk categories based on the aforementioned 8 risk factors are low (no risk factors), intermediate-1 (1 risk factor), intermediate-2 (2 or 3 risk factors), and high (4 or more risk factors), with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years. 43

Most recently, CALR mutations have been associated with favorable and ASXL1 mutations with unfavorable survival in PMF, independent of DIPSS-plus risk category; CALR<sup>−</sup> ASXL1<sup>−</sup> patients displayed the longest survival, at a median of 10.4 vs 2.3 years in CALR<sup>+</sup> ASXL1<sup>−</sup> patients vs 5.8 years in CALR<sup>−</sup> ASXL1<sup>+</sup> or CAL<sup>−</sup> ASXL1<sup>−</sup> patients. Based on these seminal observations, new prognostic models that incorporate mutational status have been devised and presented at the 2014 American Society of Hematology annual meeting. The first was referred to as MIPSS (mutation-enhanced international prognostic scoring system), 44 and the second, GPSS (genetics-based prognostic system) 45; additional details are forthcoming. Until then, the absence of type I/type 1-like CALR mutations and the presence of ASXL1 mutations define genetically high-risk disease (Figure 4). 5

Treatment

Polycythemia Vera and Essential Thrombocythemia

Survival in ET and PV is relatively long, and risk of leukemic transformation low. Current treatment has not been shown to modify these favorable outcomes. Instead, controlled clinical trials have shown increased risk of acute leukemia with use of chlorambucil in PV; 46 radiophosphorus in PV, 46 and pipobroman in PV 47 and increased risk of fibrotic transformation and arterial thrombosis with use of anagrelide hydrochloride in ET. 48 Therefore, one has to be careful in introducing new drugs for patients with PV or ET given the lack of confirmation of long-term safety and superiority over cur-
rent first-line (hydroxyurea) and second-line (interferon-alfa, busulfan) drugs of choice. This is particularly important when considering the use of ruxolitinib (a JAK inhibitor), which was recently approved for use in hydroxyurea-intolerant or resistant PV, because of (1) lack of long-term safety information, (2) lack of evidence to suggest disease-modifying activity,7 (3) emerging evidence for ruxolitinib-induced immune suppression and risk of opportunistic infections,49 and (4) the availability of alternative effective drugs (eg, interferon-alfa and busulfan) with better long-term safety information.50,51 Similarly, it is equally important not to equate occasional JAK2 or CALR mutant burden suppression by interferon-alfa52,53 or busulfan54 with markers of superior drug efficacy because the relevance of such biological activity to clinical outcomes of thrombosis and survival is not known.

The primary intent of current therapy in PV and ET should be to prevent thrombosis and alleviate symptoms. Randomized studies have shown the antithrombotic value of treatment with aspirin in PV,55 hydroxyurea in high-risk ET,56 and phlebotomy (hematocrit target <45%) in PV.57 Aspirin therapy has also been shown to be effective in alleviating microvascular symptoms, such as erythromelalgia and headaches, and possibly preventing vascular events in JAK2-mutated ET.58 Furthermore, laboratory evidence suggests that twice-daily aspirin may work better than a once-daily dose.59 On the basis of these observations, we recommend (1) phlebotomy with a hematocrit target of 45% in all patients with PV, (2) aspirin therapy (81 mg/d) in all patients with PV and JAK2-mutated ET, and (3) twice-daily aspirin use in low-risk patients whose microvascular symptoms are resistant to once-daily aspirin or whose risk of arterial thrombosis is higher but not high enough to require cytoreductive therapy (Figure 3).

In addition to these recommended treatments, high-risk patients (ie, age ≥60 years or with thrombosis history) require cytoreductive therapy. On the basis of prospective randomized56 and retrospective60 studies, our first-line cytoreductive drug of choice is hydroxyurea (Figure 3). In patients who are resistant to or intolerant of hydroxyurea, our second-line drugs of choice are interferon-alfa and busulfan; these recommendations are based on both controlled and uncontrolled single-arm studies that have demonstrated long-term safety and efficacy of these drugs in both PV and ET.32,53,61,66, among these 2 second-line drugs, we prefer the use of interferon-alfa for younger and busulfan for older patients. There is currently no controlled evidence to implicate hydroxyurea, interferon-alfa, or busulfan as being leukemogenic.32

Other aspects of management in PV and ET include acquired von Willebrand syndrome, which might accompany extreme thrombocytosis and thus require laboratory screening for ristocetin cofactor activity; use of aspirin should be avoided in the presence of
DIPSS indicates Dynamic International Prognostic Scoring System; WT, wild type.

* Outside clinical research, conventional drugs used for treatment of anemia include androgen preparations, prednisone, danazol, thalidomide, and, in the presence of del(5q), lenalidomide. Drugs used for treatment of splenomegaly and constitutional symptoms include hydroxyurea and ruxolitinib. In terms of clinical trials, we are currently most interested in potentially disease-modifying agents such as inhibitors of mutated isocitrate dehydrogenase protein and telomerase, as well as immune conjugates directed at leukemic stem cells. It is also important to confirm the potential value of another Janus kinase (JAK) inhibitor (momelotinib), currently in a phase-3 study, in improving anemia, in addition to its demonstrated efficacy in controlling splenomegaly and constitutional symptoms.

Figure 4. Contemporary Treatment Algorithm for Primary Myelofibrosis

Myelofibrosis

- Low DIPSS-plus risk (no risk factors)
- Intermediate-1 DIPSS-plus risk (1 risk factor)
- Intermediate-2 DIPSS-plus risk (2 or 3 risk factors)
- High DIPSS-plus risk (>4 risk factors)

- High genetic risk
  - CALR mutation negative and ASXL1 mutation positive

- Stem cell transplantation or investigational drug therapy

- Observation alone or hydroxyurea therapy

DIPSS indicates Dynamic International Prognostic Scoring System; WT, wild type.

- Observation alone or investigational drug therapy


Myelofibrosis

At present, the only treatment in MF that has the potential to cure the disease or prolong survival is stem cell transplantation (SCT). Transplant-related death or severe morbidity occurs in more than half of transplant recipients and therefore necessitates risk justification in the individual patient. We currently recommend SCT in DIPSS-plus high or intermediate-2–risk disease or genetically high-risk disease (ie, CALR/ASXL1+) (Figure 4). In 1 large representative study, 5-year survival was 37% for SCT from matched related and 30% from unrelated donors; the respective disease-free survival, 100-day treatment-related mortality, and grade 2 to 4 graft-vs-host disease rates were 33%, 18%, and 43% for SCT from matched related and 27%, 33%, and 40% from unrelated donors; history of splenectomy or use of reduced-intensity conditioning did not affect outcome whereas donor type and performance score did. A recent report suggests the feasibility of cord blood transplantation from unrelated donors in MF, although the number of patients involved was small and the results difficult to interpret. The potential value of JAK inhibitor therapy before transplantation is currently being investigated.

Unlike SCT, current drug therapy in MF has not been shown to be curative or disease modifying, save for controversies regarding the survival impact of ruxolitinib use. Accordingly, our current preference in high- or intermediate-2–risk patients with MF is to encourage participation in clinical trials (Figure 4). In the latter regard, we are currently most interested in potentially disease-modifying agents such as inhibitors of mutated IDH protein and telomerase, as well as immune conjugates directed at leukemic stem cells. We also believe that it is important to confirm the potential value of another JAK inhibitor (momelotinib), currently in a phase-3 study, in improving anemia, in addition to its demonstrated efficacy in controlling splenomegaly and constitutional symptoms.

Multiple JAK inhibitors have undergone clinical trials in MPN. Ruxolitinib has now been approved for use in intermediate- and high-risk MF and hydroxyurea-resistant or intolerant PV. Development of fedratinib was halted despite positive phase-3 results because of drug-associated encephalopathy. Mamloetinib and pacritinib are currently undergoing phase-3 studies, while other JAK inhibitors are at an earlier phase of development. Adverse effects for ruxolitinib include thrombocytopenia, anemia, a “cytokine rebound reaction” on drug discontinuation, and immune suppression, for momelotinib, thrombocytopenia, elevated pancreatic and liver enzymes, and treatment-emergent peripheral neuropathy; and for pacritinib, diarrhea and other gastrointestinal symptoms. In terms of efficacy, all 3 JAK inhibitors were effective in inducing spleen volume reduction (27%-40% response rate) and alleviation of constitutional symptoms. In addition, momelotinib therapy was effective in improving anemia in a substantial proportion of patients. None of these drugs affected JAK2V617F allele burden, reversed fibrosis, or induced complete remissions.

Outside clinical research, conventional drugs used for treatment of anemia include androgen preparations, prednisone, danazol, thalidomide, and, in the presence of del(5q), lenalidomide. Drugs used for treatment of splenomegaly and constitutional symptoms include hydroxyurea and ruxolitinib. In terms of clinical trials, we are currently most interested in potentially disease-modifying agents such as inhibitors of mutated isocitrate dehydrogenase protein and telomerase, as well as immune conjugates directed at leukemic stem cells. It is also important to confirm the potential value of another Janus kinase (JAK) inhibitor (momelotinib), currently in a phase-3 study, in improving anemia, in addition to its demonstrated efficacy in controlling splenomegaly and constitutional symptoms.

There is currently no evidence to support the value of specific therapy in asymptomatic low- or intermediate-1–risk disease (Figure 4). Outside the research setting, in symptomatic patients, our initial drugs of choice for MF-associated anemia are androgens, prednisone, danazol, thalidomide with or without prednisone, or lenalidomide with or without prednisone, with expected response rates of 15% to 25%. Lenalidomide works best in the presence of del(5q)1. Our first-line drug of choice for MF-associated splenomegaly is hydroxyurea. Hydroxyurea-refractory splenomegaly is often managed with ruxolitinib therapy or splenectomy. Ruxolitinib is preferred in nonsurgical candidates and splenectomy in the setting of thrombocytopenia and transfusion-dependent anemia. Involved-field radiotherapy offers temporary relief for drug-refractory organomegaly and is the treatment of choice for non-hepatosplenic extramedullary hematopoiesis.

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Conclusions

Stem cell transplantation is currently the most effective anticolonic treatment in MF but provides durable remissions in only one-third of treated patients.69 Furthermore, analysis of our institutional database suggests that more than 80% of patients with PMF would not be eligible for SCT because of advanced age, comorbidities, lack of appropriate donors, and other factors. In other words, there is an urgent need for disease-modifying drugs for more than 90% of patients with MF, and if and when such drugs become available, they will probably be equally useful in high-risk PV. For now, participation in investigational drug therapy is the most prudent treatment approach in nontransplant candidates. Palliative therapy in MPN is important and effectively accomplished by currently available conventional drugs, including ruxolitinib.71 Ongoing discoveries of mutations in MPN continue to facilitate the identification of drug targets and prognostic biomarkers that are already being used in clinical practice.13,22

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REFERENCES


19. Li J, Kent DG, Chen E, Green AR. Mouse models of JAK2(V617F) and SF3B1 mutations lead to an increased risk of polycythemia vera, essential thrombocythemia, and myelofibrosis. Blood. 2014;123(22):e123-e133.


